REMARKS/ARGUMENTS

Status of the Claims

Claims 12-19 were rejected. Claims 1-11 were previously cancelled without prejudice or disclaimer. Applicants reserve the right to pursue these claims in a continuation or divisional application. Claims 12-19 are pending in the present application.

The Rejection of the Claims Under 35 U.S.C. § 102 Should Be Withdrawn

Claims 12, 13, 15, 16, 18, and 19 were rejected under 35 U.S.C. § 102(b) as being anticipated by Nho *et al.* (U.S. Patent No. 5,234,903). This rejection is respectfully traversed.

Claims 12-19 are drawn to a method of preparing a chemically modified hemoglobin solution that is substantially free of contaminants comprising dissolving an activated aPEG in a solvent in which the aPEG is stable, filtering the aPEG solution to substantially reduce the level of contaminants, and combining the filtered aPEG solution with a hemoglobin solution. Thus, the claimed methods require that the aPEG must first be dissolved in an appropriate solvent and then filtered before using the aPEG solution to chemically modify hemoglobin. A critical element of the claimed invention is the use of a filtered aPEG solution to chemically modify hemoglobin. While contaminants such as endotoxin can be removed after PEGylation, purification following chemical modification of the hemoglobin solution results in undesirable changes to the protein composition (e.g., removal of antioxidant enzymes associated with the PHP complex) or even in destruction of the product (page 3, lines 1-3). Therefore, the use of a stable, filtered aPEG solution is critical to the production of a final hemoglobin product that is substantially free of contaminants.

A *prima facie* case of anticipation under 35 U.S.C. § 102 has not been established. According to the Federal Circuit, "anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration." *W.L. Gore & Assocs. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983). Nho *et al.* teach a method for producing a chemically modified hemoglobin solution comprising modifying a deoxygenated and reduced hemoglobin solution with an aPEG, *followed* by filtration and sterilization of the modified hemoglobin solution to remove contaminants (e.g., endotoxin). In contrast to the claimed methods, the cited reference does not teach first dissolving the aPEG in a solvent in which it is

stabile and then filtering the aPEG solution to substantially reduce contaminant levels *prior* to combining the aPEG solution with the hemoglobin solution. In fact, the cited reference teaches that the aPEG is added to the hemoglobin fraction as a powder, not as a solution as required by the present claims. Moreover, given that many aPEGs are labile in water and, as a result, prior to the present invention aPEGs were typically added to hemoglobin solutions in a powdered form, the claim limitation at issue is also not inherently disclosed by the cited reference. Nho *et al.* do not teach or suggest preparing a filtered aPEG solution and using the filtered solution to modify hemoglobin. The reference does not teach each and every element of the claims and, therefore, does not anticipate the present invention.

The Examiner maintains that Nho *et al.* do teach dissolving the aPEG in a solvent, filtering the aPEG solution, and then combining the resultant filtered aPEG solution with a hemoglobin solution. Moreover, the Examiner asserts that the filtration of the aPEG solution substantially reduces the level of contaminants, including reducing endotoxin levels to <0.1 EU. Applicants respectfully disagree with the Examiner's analysis of the cited reference.

Synthesis and activation of the aPEG taught by Nho *et al.* requires several intermediate reaction steps (see, in particular, column 14, lines 1-39). The first step in this process involves dissolving the unactivated PEG in a solvent (e.g., toluene/dichloromethane) and then drying the solution. The residue is redissolved in a solvent, activated with, for example, N-hydroxysuccinimide and triethylamine, filtered, and dried again. The resulting residue is dissolved in a solvent, "filtered from trace insolubles, and cooled to facilitate *precipitation*" of the aPEG (column 14, lines 21-22; emphasis added). The aPEG product is then collected by filtration and recrystallized as a "white powder" (column 14, line 39). It is this crystallized aPEG powder that is reacted with the hemoglobin solution to produce a chemically modified hemoglobin. Contrary to the Examiner's assertions, Nho *et al.* simply do not teach dissolving the aPEG in a solvent, filtering it to substantially reduce contaminant levels, and then combining this filtered aPEG solution with a hemoglobin solution. In fact, the steps of dissolving and filtering the Examiner refers to occur only *during the synthesis and activation of the solid aPEG* and do not equate with the production of a stable, filtered aPEG solution, as required by the present methods.

Furthermore, even if the cited reference did teach an aPEG solution, the recited filtration steps performed during synthesis of the aPEG would be insufficient to substantially reduce the level of contaminants. The filtration steps cited by the Examiner involve removal of residual chemical reactants and filtration from "trace insolubles," not the substantial reduction in contaminants required by the present claims. In fact, only *after* chemical modification is the hemoglobin solution of Nho *et al.* filtered and sterilized to substantially reduce contaminant levels. Thus, contrary to the Examiner's assertion, the reduction in endotoxin levels reported in Table IV is due to filtration and sterilization of the final hemoglobin product post-chemical modification and is not the result of filtering an aPEG solution prior to combining it with hemoglobin. The Examiner's attention is drawn to Tables 2 and 3 of the present specification which demonstrate that producing a stable aPEG solution and filtering it *prior* to combining the aPEG solution with hemoglobin results in a final chemically modified hemoglobin product that is substantially reduced in endotoxin contaminant levels, particularly when compared to the endotoxin levels of a hemoglobin solution produced by the addition of a powdered aPEG. As indicated above, the hemoglobin solution produced by the claimed methods cannot be purified after chemical modification because such filtration would disrupt, or even destroy, the hemoglobin composition. Therefore, a stable aPEG solution that can be filtered to substantially reduce contaminant levels prior to combining it with hemoglobin must be used to practice the present invention.

In summary, the claimed methods recite that the aPEG is dissolved in a solvent, filtered to substantially reduce the level of contaminants, and then the resulting filtered aPEG solution is combined with a hemoglobin solution. Filtering the aPEG solution and then using the filtered aPEG solution to modify hemoglobin are critical steps in the present methods for producing a hemoglobin solution that is substantially free of contaminants. Moreover, *prior to the present disclosure it was not known that a stable aPEG solution could be produced, filtered to substantially reduce contaminant levels, and successfully used to modify a hemoglobin solution.* The cited reference teaches synthesizing an aPEG powder, combining the solid aPEG with hemoglobin, and filtering the hemoglobin solution *after* chemical modification to remove endotoxin and other contaminants from the final product. Nho *et al.* do not teach the critical steps of preparing a stable aPEG solution and filtering the aPEG solution to substantially reduce

contaminant levels prior to combining the filtered solution with a hemoglobin solution. Accordingly, the reference does not teach each and every element of the claims, and a *prima facie* case of anticipation under 35 U.S.C. § 102 has not been established.

The Rejection of the Claims Under 35 U.S.C. § 103 Should Be Withdrawn

Claims 14 and 17 were rejected under 35 U.S.C. § 103 as being unpatentable over Nho *et al.* in view of Woghiren *et al.* (1993) *Bioconj. Chem.* 4:314-318. This rejection is respectfully traversed.

Dependent claim 14 further requires that the solvent for the aPEG is selected from the group consisting of ethanol, methanol, and acetonitrile. The Examiner maintains that while Nho *et al.* do not expressly teach methanol as a solvent for dissolving the aPEG, "the use of an alternative solvent such as methanol to dissolve the activated PEG was well known in the art" (page 4, Office Action mailed November 16, 2004). In particular, the Examiner states that Woghiren *et al.* teach the use of methanol as a solvent for dissolving an aPEG. The Examiner concludes that it would have been obvious to one skilled in the art to "use an alternative solvent such as Woghiren's methanol in place of Nho's toluene to produce the instant invention with a reasonable expectation of success" (page 4, Office Action mailed November 16, 2004). Applicants respectfully disagree with the Examiner's conclusions.

First, as discussed above, Nho *et al.* do not teach dissolving the aPEG in any solvent, filtering the aPEG solution, and combining the filtered solution with hemoglobin. In fact, this reference describes using a solvent during the synthesis of a powdered aPEG. Furthermore, Woghiren *et al.* also do not disclose dissolving an aPEG in methanol. The cited reference teaches the use of methanol in an alcoholysis step during the synthesis of PEG-SH, not for the dissolution of the final aPEG as the Examiner suggests. Neither of the cited references teaches dissolving an aPEG in a solvent and filtering the aPEG solution to substantially reduce contaminant levels prior to using the filtered solution to modify a protein solution.

A *prima facie* case of obviousness requires some suggestion to combine the cited references to arrive at the claimed invention and a reasonable expectation of success in such a combination. In the present case, even if the references are combined, the references would not allow one of skill in the art to practice the claimed method. As discussed above, neither

reference cited by the Examiner teaches dissolving an aPEG in a solvent, filtering the aPEG solution to substantially reduce contaminant levels, and combining the filtered aPEG solution with hemoglobin, critical steps in the present method. Prior to the present disclosure it was not known that a stable aPEG solution could be produced, filtered, and successfully used to produce a chemically modified hemoglobin that is substantially free of contaminants. Accordingly, the combination of cited references could not have placed the invention of claim 14 in the hands of the public, and a *prima facie* case of obviousness has not been established.

The Examiner further asserts that claim 17 is obvious in view of Nho *et al.* Dependent claim 17 further comprises filtering the aPEG solution through a 0.2 micron nylon filter. Nho *et al.* teach using a 0.2 micron Zetapor® nylon filter to sterilize the final chemically modified hemoglobin solution and to render it substantially endotoxin-free. In light of this, the Examiner maintains that it would have been obvious to one of skill in the art to use a 0.2 micron nylon filter to filter an aPEG solution to substantially reduce contaminant levels. As indicated above, Nho *et al.* do not teach or suggest dissolving an aPEG in a solvent, filtering the aPEG solution through a filter of any size, and using the filtered aPEG solution to modify a hemoglobin solution. The aPEG used by Nho *et al.* is a powder, not a solution and, therefore, is not filtered. Moreover, the plastic housing and the O-ring components of the 0.2 micron Zetapor® nylon filter make this filter incompatible with filtration of organic solvents (e.g., methanol, toluene/dichloromethane, etc.) as the Examiner proposes. And finally, the mere fact that Nho *et al.* use a 0.2 micron nylon filter to remove contaminants from a chemically modified hemoglobin solution is no indication that one of skill in the art would have been motivated to dissolve an aPEG in a solvent in which it is stable, filter the aPEG solution through a filter of any size, and then combine the filtered aPEG solution with a hemoglobin solution. Therefore, claim 17 is not obvious in view of the cited reference.

For the reasons presented above, the Examiner has failed to establish a *prima facie* case of obviousness. Accordingly, Applicants respectfully submit that the claimed methods for producing a chemically modified hemoglobin solution that is substantially free of contaminants are not obvious in view of the cited references and request that the rejection of claims 14 and 17 under 35 U.S.C. § 103(a) be withdrawn.

CONCLUSION

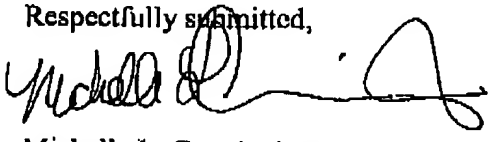
The Examiner is respectfully requested to withdraw the rejections and allow claims 12-19. In any event, the Examiner is respectfully requested to consider the above remarks for the purposes of further prosecution.

Accordingly, in view of the above remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,



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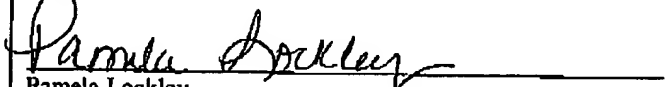

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